EFFECTS OF CYTOCHROME P-450 INHIBITORS AND INDUCERS ON CCL₄ TOXICITY IN PRIMARY HEPATOCYTE CULTURES FROM MONGOLIAN GERBILS

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Abstract: The influence of the incubation period and the effects of cytochrome P-450 (CYP) inhibitors and inducers on CCl₄ toxicity in gerbil hepatocyte cultures were studied. The prolonged incubation of gerbil hepatocyte cultures after seeding was less sensitive to CCl₄-induced toxicity and affected the immunostainability of some CYP apoproteins. SKF-525A and chloramphenicol (CPC), which are CYP inhibitors, had significant protective effects on the CCl₄-induced T-Chol increase and enzyme leakage. 3-Methylcholanthrene (3-MC) and clofibrate (CFA) enhanced the CCl₄ toxicity, increasing LDH leakage and intracellular contents of TG and T-Chol. 3-MC and CFA treated cells were intensely positive for multi-isoform of CYPs. Ethanol (EtOH) also enhanced the CCl₄-induced enzyme leakage and cytoplasmic vacuolation without fatty changes, and increased only CYP2E1 staining level within cells. This study revealed that CCl₄-induced toxicity in gerbil hepatocytes also depends on some CYP activities. (J Toxicol Pathol 9: 329~339, 1996)

Key words: CCl₄, Hepatotoxicity, Mongolian gerbil, Cytochrome P-450 isozyme

Introduction

The hepatotoxicity of the halomethanes, CCl₄ and CHCl₃, is believed to depend on cytochrome P-450 (CYP) producing reactive metabolites such as trichloromethyl radicals and phosgene¹. Difference in susceptibility to the hepatotoxic agents among animal species might reflect the ability of biotransformation of chemicals to reactive metabolites covalently binding with the cell membrane. Induction of the CYP activities by agents such as phenobarbital, polychlorinated biphenyls or ethanol increases the extent of CCl₄ metabolism, resulting in enhancement of its hepatotoxicity²⁻⁴. On the other hand, 2-diethylaminoethyl-2, 2-diphenyl-n-pentanoate hydrochloride (SKF-525A) or chloramphenicol (CPC) can inhibit CYP-mediated CCl₄ bioactivation and results

in lowered hepatotoxicity⁵.

The Mongolian gerbil is known to have higher CYP levels in hepatic microsomes than the rat, and has different CYP dependent monooxygenase activities^{6,7}. The gerbil is very sensitive to CCl₄ hepatotoxicity^{8,9}, showing periportal or diffuse fatty degeneration of liver cells, whereas centrilobular necrosis is less intense¹⁰. Cai and Mehendale¹¹ reported that none of chlordecone, phenobarbital (PB), or mirex affected hepatotoxic and lethal effects of CCl₄ in the gerbil. However, we showed that PB potentiated the necrotic changes of hepatocytes *in vivo* and *in vitro*¹⁰.

The cultured hepatocytes loses many metabolic and detoxicating activities for chemicals depending on incubation time^{12,13}. After 24 to 48 hr of culture, the CYP level in adult rat hepatocytes reaches 20 to 30% of the initial level with decreasing monooxygenase activities^{13,14}. On the other hand, some CYP inducers can increase the synthesis of specific mRNAs and proteins of CYPs with enhanced corresponding catalytic activities in the primary cultured rat hepatocytes^{15,16}.

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The present study is to see the influence of the culture period and the effects of CYP inhibitors or inducers on susceptibility of gerbil hepatocyte cultures to CCl₄ toxicity.

Materials and Methods

Chemicals

CCl₄, dimethyl sulfoxide (DMSO), 3-methylcholanthrene (3-MC), sodium phenobarbital (PB), and ethanol (EtOH) were purchased from Wako Pure Chemical Industries (Osaka). Chloramphenicol (CPC), isonicotinic acid hydrazide (isoniazid; INH), ethyl-2-(4-chlorophenoxy)-2-methylpropionic acid (clofibric acid; CFA), dexamethasone, insulin, and trypsin inhibitor were purchased from Sigma Chemical (St Louis, MO). 2-diethylaminoethyl-2,2-diphenyl-n-pentanoate hydrochloride (SKF-525A) was from Research Biochemical (MN). William's E medium and swine serum were from ICN Biomedicals (UK) and Nippon Bio-Supply Center (Tokyo), respectively. Collagenase type I was from Yakult (Tokyo).

Hepatocyte cultures

Hepatocytes from TUM-line male Mongolian gerbils aged 8 to 12 weeks were prepared by two-step collagenase perfusion method describing previously¹⁰. Briefly, the liver of deeply anesthetized gerbils were perfused via the portal vein with preperfusion buffer (137 mM NaCl, 4mM NaHCO₃, 0.5 mM NaH₂PO₃, 0.4 mM Na₂HPO₃, 10 mM HEPES, 0.5 mM EGTA), and then with collagenase solution (50 mU/ml collagenase, 50 µg/ml trypsin inhibitor, 137 mM NaCl, 4 mM NaHCO₃, 0.5 mM NaH₂PO₃, 0.4 mM Na₂HPO₃, 5 mM CaCl₂, 10 mM HEPES). Thereafter, the liver was gently minced and filtered through a stainless mesh, and cells were collected by centrifugation at 40×g for 90 sec and washed by centrifugation 3 to 5 times with William's E medium. The cells were suspended at a density of 4×10⁵ cells/ml in incubation medium (William's E medium containing 10% porcine serum, 4 ng/ml dexamethasone, 5µg/ml insulin). Cells (8×10⁵ or 4×10⁶) were introduced into collagen coated 35 mm-dishes or 2-well chamber slides (Nunc, Naperville, IL), respectively, and incubated in a humidified atmosphere of 95% air and

5% CO₂ at 37°C. The culture medium was replaced 3 hr later and thereafter once a day.

Biochemical assay

The hepatocytes cultured for 18 to 24 hr were incubated with SKF-525A (10 µM) or CPC (100 µM) for 6 hr to inhibit CYP activities. To induce CYPs, cells were incubated with 3-MC (100 µM), PB (1mM), INH (100 µM), EtOH (1mM), and CFA (200 µM) for 72 hr. The cultures were then exposed to CCl₄ for 24 hr. CPC, 3-MC, INH, CFA, and CCl₄ were dissolved in DMSO, while SKF-525A and PB in saline and EtOH in distilled water. The CYP inducers were diluted with incubation medium, and SKF-525A, CPC, and CCl₄ solutions were diluted with serum free medium. To the vehicle control dishes, the amount of DMSO or saline corresponding to the solvent at the highest concentration of chemicals.

The incubation medium and sonicated cell suspensions were stored at -20°C, until assay for LDH or AST activity according to Wroblewski or Reitman and Franckel method. Ultrasonicated cell suspensions were assayed for triglyceride (TG), total cholesterol(T-Chol), and phospholipids (LP) by the LPL-GK-GPO, CHE-COD, and POD-COD methods, respectively, using commercial kits (Wako Pure Chemicals, Osaka). The protein content of the cell suspension was estimated by a commercial BCA protein assay kit using bovine serum albumin as a standard.

Immunocytochemistry

Cells on chamber slides were washed in 10 mM phosphate buffered saline (pH 7.2, PBS), and fixed in 4% paraformaldehyde-100 mM phosphate buffer (pH 7.2, PB) for 1 hr at 4°C. After washing in PBS for 1 hr, the slides were dipped in 0.3% hydrogen peroxide (H₂O₂)-methanol solution to inactivate endogeneous peroxidase. After three washing for 5 min in PBS, they were incubated in PBS containing 4% skim milk for 20 min to reduce the non-specific background. They were incubated with a 1:400 dilution of primary goat antibodies to each rat hepatic CYP isozymes for 2 hr, and then with biotinylated anti-goat IgG rabbit IgG for 30 min and streptavidin coupled to peroxidase for 30 min. The slides were colored with 0.03% 3, 3'-diaminobenzidine (DAB) in 50mM

Tris-HCl buffer (pH 7.6) for 1 to 3 min. and counter-stained with methyl green. The primary goat polyclonal antibodies against rat CYP1A1, CYP2B1, CYP2E1, CYP3A2, and CYP4A1, which had affinity for rat cytochrome P-450 IA1/2, IIB1/2, IIE1, IIIA2, and VIA1 subfamilies, respectively, were obtained from Daiichi Pure Chemicals (Tokyo). The biotinylated rabbit IgG to goat IgG and peroxidase conjugated streptavidin were from a commercial kit (HISTOFINE; Nichirei, Tokyo).

Statistical analysis

Differences in the mean values of three or four experiments in enzyme leakage and lipid contents of gerbil hepatocytes compared with the control group (CCl₄-alone treated group) were analyzed by Student's *t* test.

Results

Twenty-four- and 48 hr-cultures of gerbil hepatocytes showed remarkable cellular LDH leakage after exposure to 0.5 mM or more CCl₄, that of the 72 hr-culture was lower and there was none from the 96 hr-culture (Fig. 1). As presented in Fig. 2, the 24 and 48 hr-cultures had augmented cellular TG content after CCl₄ exposure, but there was no significant increase of TG in the 96 hr-culture. In T-Chol content, the 48 hr-culture had less than the 24 hr-culture and the level in the 96 hr-culture remained at

Fig. 1. LDH leakage from gerbil hepatocytes exposed to CCl₄ at different times after seeding. Hepatocytes cultured for 24 to 96 hr were exposed to 0.5 to 4 mM CCl₄ for 24 hr. Each value represents mean (*n*=5).

the control level. Phase contrast microscopy (PCM) revealed that lipid changes and cell debris caused by CCl₄ exposure was not so severe in 96 hr-cultures as compared with 24 hr-cultures (Fig. 3A and B).

Immunocytochemically a large number of 24 hr-cultured hepatocytes were moderately positive for CYP2B1, CYP2E1, and CYP3A2 (Table 1, Figs. 4A to C), whereas a few cells were mildly positive for CYP1A1 and CYP4A1. In 96 hr-cultures, however, there were less CYP2B1 positive cells in both number and staining intensity (Fig. 4D) and there were few CYP3A2 positive cells (Fig. 4F), CYP2E1 positive cells remained with the same staining intensity (Fig.

Fig. 2. Lipid content in gerbil hepatocytes exposed to CCl₄ at different times after seeding. Hepatocytes cultured for 24 to 96 hr were exposed to 0.5, 1 and 2 mM CCl₄ for 24 hr. Each value represents mean (*n*=5).

Fig. 3. Phase contrast micrographs of CCl₄ toxic changes in cultured gerbil hepatocytes following exposure to various chemical inducers. (A) 24-hr culture, (B) 96-hr culture, (C) 3-MC, (D) EtOH, (E) CFA. $\times 300$.

Table 1. Immunocytochemistry of CYP Isozymes in 24- and 96-hr Cultures of Gerbil Hepatocytes

Culture time (hr)	P-450 isozyme				
	CYP1A1	CYP2B1	CYP2E1	CYP3A2	CYP4A1
24	+	#	#	#	+
96	-	±	+	±	±

Hepatocyte monolayers cultured for 24 and 96 hr were stained immunocytochemically (SAB method) using anti-rat hepatic CYP isozymes antibodies.

Fig. 4. Immunocytochemistry for CYP isozymes in cultured gerbil hepatocytes at 24- (A-C) and 96-hr (D-F) after seeding: CYP2B1 (A, D), CYP2E1 (B, E), CYP3A2 (C, F) $\times 150$.

4E). There was an increase of CYP1A1 and CYP4A1 negative cells.

The 18- to 24 hr-cultures of gerbil hepatocytes were treated with 10 μ M SKF-525A or 100 μ M CPC for 6 hr, and then exposed to 0.5mM CCl_4 for 24 hr. Both SKF-525A and CPC mildly reduced AST leakage from the hepatocytes, whereas they did not reduce LDH leakage (Fig. 5). Both chemicals significantly inhibited the T-Chol increase ($p < 0.05$), but had no effect on the TG increase (Table 2).

The 24 hr-cultured gerbil hepatocytes were treated with CYP inducers, 100 μ M 3-MC, 1mM PB, 100

μ M INH, 1mM EtOH or 200 μ M CFA, for 72 hr prior to 0.5 mM CCl_4 . As shown in Figs. 6 and 7, LDH leakage as well as the increases of cellular TG and T-Chol were significantly enhanced by 3-MC and CFA in gerbil hepatocytes exposed to CCl_4 , as compared with those exposed to CCl_4 treatment only. EtOH also significantly enhanced the LDH leakage caused by CCl_4 , but it suppressed the increase of cellular lipid contents. PB slightly increased the LDH leakage. INH had little effect on LDH leakage or the lipid contents after CCl_4 exposure. Remarkable increase in size and number of lipid droplets and

Fig. 5. Effects of SKF-525A or chloramphenicol (CPC) on enzyme (LDH or AST) leakage from cultured gerbil hepatocytes exposed to CCl₄. Cultured hepatocytes were incubated with 10 μ M SKF or 100 μ M CPC for 24 hr then with 0.5 mM CCl₄. Enzyme leakage into the medium was determined 24 hr later and is expressed as % of the total activity. Each value is mean $\pm$ SEM of three experiments.

* $p < 0.05$, ** $p < 0.01$: significantly different from CCl₄ alone (Student's *t*-test).

vacuoles within 3-MC and CFA treated cells was observed by PCM after exposure to CCl₄ (Figs. 3C and E). EtOH-treated cells showed severe cytoplasmic vacuolation (Fig. 3D), but PB and INH showed only slight cytomorphologic changes.

As shown in Table 3, gerbil hepatocytes treated

Fig. 6. Effects of CYP inducers on CCl₄ induced LDH leakage from gerbil hepatocytes. Primary monolayer cultures were incubated with CYP inducers (100 μ M 3-MC, 1 mM PB, 1 mM EtOH, 100 μ M INH, or 200 μ M CFA) for 72 hr prior to 0.5 mM CCl₄ exposure. LDH leakage was determined 24 hr after CCl₄ exposure. Values represent mean $\pm$ S.D. (n=4)

* $p < 0.05$, ** $p < 0.01$: significantly different from CCl₄ alone (Student's *t*-test).

with 3-MC, PB, EtOH, and CFA were highly or moderately positive for CYP1A1, CYP2B1, CYP2E1, and CYP4A1 immunostaining, respectively (Fig. 8). 3-MC and CFA treatment remarkably increased the

Table 2. Effects of SKF-525A or Chloramphenicol (CPC) on Cellular TG, T-Chol, PL Contents in Cultured Gerbil Hepatocyte Exposed to CCl₄

Compound	CCl ₄ (mM)	Lipid content (μ g/mg protein)		
		TG	T-Chol	PL
—	0	197.4 $\pm$ 25.9** (100%)	72.0 $\pm$ 21.3** (100%)	115.2 $\pm$ 12.2 (100%)
	0.5	369.2 $\pm$ 68.6 (191%)	142.4 $\pm$ 26.9 (228%)	143.3 $\pm$ 4.6 (115%)
SKF-525A	0	220.2 $\pm$ 20.5** (114%)	75.0 $\pm$ 22.3** (103%)	133.4 $\pm$ 15.6 (116%)
	0.5	304.6 $\pm$ 38.0 (156.%)	104.1 $\pm$ 26.4* (149%)	131.1 $\pm$ 3.7 (115%)
CPC	0	206.9 $\pm$ 26.7** (105%)	78.6 $\pm$ 18.1** (115%)	129.7 $\pm$ 7.8 (114%)
	0.5	369.2 $\pm$ 68.6 (157%)	82.5 $\pm$ 15.2* (127%)	128.1 $\pm$ 18.9 (110%)

Hepatocytes were incubated with 10 μ M SKF-525A or 100 μ M CPC for 24 hr, then with 0.5 mM CCl₄. The lipid content was determined 24 hr later. Results are the mean $\pm$ SEM of the determinations of three experiments and represent the mean % of each group value compared with the non-treated control value in parenthesis.

* $p < 0.05$, ** $p < 0.01$; Significantly different from CCl₄ alone (Student's *t*-test)

Fig. 7. Effects of CYP inducers on CCl_4 induced lipid changes of gerbil hepatocytes. Primary monolayer cultures were treated with CYP inducers ($100 \mu\text{M}$ 3-MC, 1 mM PB, 1 mM EtOH, $100 \mu\text{M}$ INH, or $200 \mu\text{M}$ CFA) for 72 hr prior to 0.5 mM CCl_4 exposure. Lipid contents were determined 24 hr after CCl_4 exposure. Values represent mean $\pm$ S.D. ($n=4$)

* $p < 0.05$, ** $p < 0.01$; significantly different from CCl_4 alone (Student's t -test).

Table 3. The Profile of CYP Isozymes Identified by Immunocytochemistry in Cultured Gerbil Hepatocytes Following Exposure to Various Chemical Inducers

P-450 inducers	P-450 isozyme				
	CYP1A1	CYP2B1	CYP2E1	CYP3A2	CYP4A1
Control	—	±	+	±	±
3-MC	±	+	±	±	±
PB	±	±	+	±	±
INH	+	±	+	±	±
EtOH	—	+	±	±	+
CFA	±	±	±	±	±

Primary monolayer cultures were exposed to DMSO (Control, 0.1%), 3-methylcholanthrene (3-MC, $100 \mu\text{M}$), phenobarbital (PB, 1 mM), isonicotinic acid hydrazide (INH, $100 \mu\text{M}$), ethanol (EtOH, 1 mM), and chlofibric acid (CFA, $200 \mu\text{M}$) for 72 hr. The cell monolayers were fixed in 4% paraformaldehyde (1 hr) and stained immunocytochemically (SAB) using anti-rat hepatic CYP isozymes antibodies.

intensity and number of cells positive for both CYP2E1 and CYP3A2 (Fig. 9). Furthermore, CFA-treated cells were highly and moderately positive for CYP1A1 and CYP2B1, respectively. INH treated cells showed mildly enhanced stainability for CYP1A1 and CYP2E1.

Discussion

In the primary rat hepatocyte cultures, the con-

tent and catalytic activities of CYP markedly decline with the incubation period, and the ability of metabolism or detoxication of chemicals decreases significantly^{12,13}. The CYP content in gerbil hepatocytes at 24 hr was 40% of the original value, showing loss of the activity of 7-ethoxycoumarin *O*-deethylase or coumarin 7-hydroxylase, whereas activities of erythromycin and benzphetamine *N*-demethylase and *p*-nitrophenol hydroxylase were maintained¹⁷. The 24 hr-culture of gerbil he-

Fig. 8. Immunocytochemistry for CYP isozymes in cultured gerbil hepatocytes following exposure to various chemical inducers. : CYP1A1 (A ; Control, E ; 3-MC), CYP2B1 (B ; Control, F ; PB), CYP2E1 (C ; Control, G ; EtOH), CYP4A1 (D ; Control, H ; CFA), $\times 150$.

patocytes, which were sensitive to CCl₄, were moderately positive for CYP2B1, CYP2E1, and CYP3A2 and mildly for CYP1A1 and CYP4A1. At 96 hr,

the cells were much less susceptible to CCl₄ and showed remarkably declined level of CYP3A2 and CYP2B1, while CYP2E1 reduced slightly. CCl₄ is

Fig. 9. Immunocytochemistry for CYP2E1 and CYP3A2 in cultured gerbil hepatocytes following exposure to 3-MC or CFA. CYP2E1 (A; 3-MC, C; CFA), CYP3A2 (B; 3-MC, D; CFA), $\times 150$.

cleaved by CYP2E1, producing highly reactive trichloromethyl radicals and lipid peroxidation in the endoplasmic reticulum, resulting in cell injury¹⁸. On the other hand, cultured rat hepatocytes selectively lose CYP2E and CYP3A immunoreactive from 72 to 96 hr^{19,20}. These findings suggested that the CCl_4 toxicity in gerbil hepatocytes is dependent on some CYPs activities; not only CYP2E1 but also CYP3A2.

Alkylamine derivatives, such as SKF-525A, can be converted by CYP to reactive intermediates that sequester the cytochrome in a catalytically inactive complex state^{21,22}. On the other hand, CPC has been reported to elicit the autocatalytic destruction of P-450^{23,24}, and exerts its inhibitory effect on mixed-function oxidase activity by CYP modification rather than by heme destruction. Both SKF-525A and CPC significantly protected gerbil hepatocytes against the CCl_4 -induced T-Chol increase, thus inhibiting AST leakage. Both compounds are not uniformly potent against the activity of all CYP. SKF-525A generates complexes with CYP2B1, CYP2C11, and

CYP3A1/2 *in vivo*, but not with CYP2A1²⁵. CPC inactivates rat hepatic CYP2C6, CYP2C11, and CYP2B1, whereas CYP1A1/2, and CYP4A are resistant to inactivation²³. Thus CCl_4 -induced toxic changes might be caused by CYP catabolic activities of specific P-450 isozymes.

Fentem *et al.*¹⁷ reported that hepatocyte cultures from PB-treated gerbils had increased levels of total CYP content and of 7-ethoxycoumarin *O*-deethylase, *p*-nitrophenol hydroxylase, and coumarin 7-hydroxylase activities, and they were highly sensitive to precocene II toxicity mediated by CYP. Some CYP inducers can enhance one or a few specific CYP isozymes in cultured hepatocytes^{15,16}.

3-MC induces CYP1A1 in the rat liver²⁶ as well as in cultured hepatocytes²⁷. CFA activates the induction of CYP4A1 protein and mRNA *in vivo* and *in vitro*^{28,29} and can induce CYP2B and CYP3A isozymes in cultured hepatocytes¹⁵. In gerbil hepatocytes, both 3-MC and CFA induced not only CYP1A1 and/or CYP4A1, but also CYP2B1, CYP2E1 and/or CYP3A2. The cells treated with 3-

MC and CFA were more sensitive to CCl₄ toxicity, as evidenced by remarkable fatty changes and vacuolation with enzyme leakage. 3-MC and CFA were inducible multiple CYP isozymes in gerbil hepatocytes, resulting in enhancing the susceptibility of gerbil hepatocytes to CCl₄ toxicity.

EtOH, inducing CYP2E1 with CYP2B1/2 and CYP3A in the rat hepatocytes³⁰, also augmented CCl₄ toxicity in gerbil hepatocytes caused enzyme leakage and cytoplasmic vacuolation but no fatty changes. In the EtOH-treated cells, there was an increment of CYP2E1 but no other isozymes. Therefore, in gerbil hepatocytes, CYP2E1 was related to membranous cell damage by CCl₄, but not to fatty change. CYP2E1 is induced by INH in the rat liver³¹, but not in cultured rat hepatocytes¹⁶. In the gerbil hepatocytes, INH slightly induced CYP1A1 and CYP2E1 without significantly enhancing CCl₄ toxicity.

PB increases the *de novo* synthesis of apocytochrome CYP2B1/2 and CYP3A in the rat liver or hepatocyte cultures^{15,32}. Wortelboer *et al.*¹⁶ reported that either PB or CFA induced CYP2B1/2 mRNA and the apoprotein level with the associated enzyme activities in cultured rat hepatocytes, although PB was less effective than *in vivo*. PB-treated gerbil hepatocytes showed only moderate induction of CYP2B1/2 and slightly enhanced cellular vacuolization after exposure to CCl₄, suggesting that the CYP2B1/2 is less effective in the production of CCl₄ toxicity in gerbil hepatocytes.

This study revealed that CCl₄-induced toxic changes in gerbil hepatocytes depended on some CYP activities. The gerbil hepatocytes intensely positive for CYP2E1 were severely injured by CCl₄ exposure, a likely result of enzyme leakage and cytoplasmic vacuolation. The CYP2E1 might produce highly reactive free radicals from CCl₄, thus damaging the cellular membrane by lipid peroxidation. On the other hand, fatty changes were inducible in cells intensely positive for CYP3A2 after CCl₄ exposure, but inhibited by SKF-525A, that generates complexes with the CYP3A1/2. These findings suggested that CYP3A2 is involved in the CCl₄-induced fatty changes in gerbil hepatocytes. However, the antibody to CYP3A2 used here cross-reacts with CYP2C11³³. SKF-525A, and CPC, both capable of inactivating CYP2C11, inhibited fatty changes in gerbil hepatocytes exposed to the CCl₄, indicating

that this isozyme might play some role in CCl₄ toxicity in gerbil hepatocytes, especially fatty changes.

References

1. MacDonald, TL: Chemical mechanisms of halocarbon metabolism. *CRC Crit Rev Toxicol* **11**: 85-120, 1983.
2. Kluwe, WM, Hook, JB, and Bernstein, J: Synergistic toxicity of CCl₄ and several aromatic organohalide compounds. *Toxicology* **23**: 321-336, 1982.
3. Lindros, KO, Cai, Y, and Penttilä, PE: Role of ethanol-inducible carbon tetrachloride induced damage to centrilobular hepatocytes from ethanol-treated rats. *Hepatology* **19**: 1092-1097, 1990.
4. Tuchweber, B, Werringloer, J, and Kourounakis, P: Effect of phenobarbital or pregnenolone-16- α -carbonitrile (PCN) pretreatment on acute CCl₄ hepatotoxicity in rats. *Biochem Pharmacol* **23**: 513-518, 1974.
5. Castro, JA, de Ferreya, EC, de Castro, CR, and de Fenos, OM: Prevention of CCl₄-induced necrosis by inhibitors of drug metabolism—Further studies on their mechanisms of action. *Biochem Pharmacol* **23**: 295-302, 1974.
6. Dominguez, K, Fentem, JH, Garle, MJ, and Fry, JR: Comparison of Mongolian gerbil and rat hepatic microsomal monooxygenase activities: high coumarin 7-hydroxylase activity in the gerbil. *Biochem Pharmacol* **39**: 1629-1631, 1990.
7. Fentem, JH, and Fry, JR: Comparison of the effects of inducers of cytochrome P450 on Mongolian gerbil and rat hepatic microsomal monooxygenase activities. *Xenobiotica* **21**: 895-904, 1991.
8. Ebel, RE, and McGrath, EA: CCl₄-hepatotoxicity in the Mongolian gerbil: Influence of monooxygenase system induction. *Toxicol Lett* **22**: 205-210, 1984.
9. de Perez, OA, de Ferreya, EC, Bernacchi, AS, Villarruel, MC, de Fenos, OM, and Castro, JA: Carbon tetrachloride-induced liver cell injury in the Mongolian gerbil (*Meriones unguiculatus*). *Comp Biochem Physiol* **94c**: 357-364, 1989.
10. Yamaguchi, I, Tsutsui, S, Mizutani, K, Yoshino, M, Ohmura, M, Takeuchi, T, and Fujiwara, K: *In vivo* and *in vitro* studies on CCl₄-induced hepatotoxicity in mongolian gerbils (*Meriones unguiculatus*). *J Toxicol Pathol* **7**: 51-59, 1994.
11. Cai, Z, and Mehendale, HM: Lethal effects of CCl₄ and its metabolism by gerbils pretreated with chlordecone, phenobarbital and mirex. *Toxicol Appl Pharmacol* **104**: 511-520, 1990.
12. Guzelian, PS, Bissel, DM, and Meyer, VA: Drug metabolism in adult rat hepatocytes in primary monolayer culture. *Gastroenterology* **72**: 1232-1239, 1977.
13. Guguen-Guillouzo, C, and Guillouzo, A: Modulation of functional activities in cultured rat hepatocytes. *Mol Cell Biochem* **53/54**: 35-56, 1983.
14. Grant, MH, Melvin, MAL, Shaw, P, Melvin, WT, and Burke, MD: Studies on the maintenance of cytochromes P-450 and b5, monooxygenases and cytochrome reductases in primary cultures of rat hepatocytes. *Bio-*

- chem J **239** : 785-788, 1985.
15. Bars, GB, Mitchell, AM, Wolf, CR, and Elcombe, CR : Induction of cytochrome P-450 in cultured rat hepatocytes: The heterogenous localization of specific isozymes using immunochemistry. *Biochem J* **262** : 151-158, 1989.
 16. Worthelboer, HM, De Kruif, CA, Van Iersel, AAJ, Falke, HE, Noordhoek, J, and Blaauwouwer, BJ : Comparison of cytochrome P450 isozyme profile in rat liver and hepatocyte cultures: The effects of model inducers on apoproteins and biotransformation activities. *Biochem Pharmacol* **42** : 381-390, 1991.
 17. Fentem, JH, Hammond, AH, and Fry, JR : Maintenance of monooxygenase activities and detection of cytochrome P-450-mediated cytotoxicity in Mongolian gerbil hepatocyte cultures. *Xenobiotica* **21** : 1363-1370, 1991.
 18. Recknagel, RO, and Glende, EAJr : Carbon tetrachloride hepatotoxicity: An example of lethal cleavage. *CRC Crit Rev Toxicol* **2** : 263-297, 1973.
 19. Hunt, CM, Guzelian, PS, Molowa, DT, and Wright, SA : Regulation of rat hepatic cytochrome P450IIE1 in primary monolayer hepatocyte culture. *Xenobiotica* **21** : 1621-1631, 1991.
 20. MacMillan, JM, Shaddock, JG, Caciano, DA, Arlotto, MP, and Leakey, EA : Differential stability of drug-metabolizing enzyme activities in primary rat hepatocytes, cultured in the absence or presence of dexamethasone. *Mut Res* **249** : 81-92, 1991.
 21. Beuning, MK, and Franklin, MR : SKF 525-A inhibition, induction, and 452nm complex formation. *Drug Metab Dispos* **4** : 244-255, 1976.
 22. Mansuy, D, Rouer, E, Bacot, C, Gans, P, Chottard, JP, and Leroux, JP : Interaction of aliphatic N-hydroxylamines with microsomal cytochrome P-450: nature of the different derived complexes and inhibitory effects on monooxygenases activities. *Biochem Pharmacol* **27** : 1229-1237, 1978.
 23. Halpet, JR, Balfour, C, Miller, NE, Morgan, ET, Dunbar, D, and Kaminsky, LS : Isozyme selectivity of the inhibition of rat liver cytochrome P-450 by chloramphenicol *in vivo*. *Mol Pharmacol* **28** : 290-296, 1985.
 24. Halpet, JR, Miller, NE, and Gorsky, LD : On the mechanism of the inactivation of the major phenobarbital-inducible isozyme of the rat liver cytochrome P-450 by chloramphenicol. *J Biol Chem* **260** : 8397-8403, 1985.
 25. Murray, M : Isozyme-selective complexation of cytochrome P-450 in hepatic microsomes from SKF 525-A-induced rats. *Arch Biochem Biophys* **262** : 381-388, 1988.
 26. Baron, J, Redick, JA, and Guengerich, FP : Effects of 3-methylcholanthrene, β -naphthoflavone, and phenobarbital on the 3-methylcholanthrene-inducible isozyme of cytochrome P-450 with centrilobular, midzonal, and periportal hepatocytes. *J Biol Chem* **25** : 953-957, 1982.
 27. Jauregui, HO, Gann, KL, and Waxman, DJ : Xenobiotic induction of P-450 PB-4 (IIB) and P-450c (IA1) and associated monooxygenase activities in primary cultures of adult rat hepatocytes. *Xenobiotica* **21** : 1091-1100, 1991.
 28. Bars, RG, Bell, DR, and Elcombe, CR : Induction of cytochrome P-450 and peroxisomal enzymes by clofibrate *in vivo* and *in vitro*. *Biochem Pharmacol* **45** : 2045-2053, 1993.
 29. Bell DR, and Elcombe CR : Induction of acyl-CoA oxidase and cytochrome P450 IVA1 RNA in rat primary hepatocyte culture by peroxisome proliferators. *Biochem J* **280** : 249-253, 1991.
 30. Sinclair, JF, McCaffrey, J, Sinclair, PR, Bement, WJ, Lambrecht, LK, Wood, SG, Smith, EL, Schenkman, JB, Guzelian, PS, Park, SS, and Gelboin, HV : Ethanol increases cytochromes P450IIE, IIB1/2, and IIIA in cultured rat hepatocytes. *Arch Biochem Biophys* **284** : 360-365, 1991.
 31. Rayan, DE, Ramanathan, L, Iida, S, Thomas, PE, Haniu, M, Shively, JE, Lieber, CS, and Levin, W : Characterization of a major form of rat hepatic microsomal cytochrome P-450 induced by isoniazid. *J Biol Chem* **260** : 6385-6393, 1985.
 32. Newman, S, and Guzelian, PS : Stimulation of de novo synthesis of cytochrome P450 by phenobarbital in primary non proliferating cultures of adult rat hepatocytes. *Proc Natl Acad Sci USA* **79** : 2922-2926, 1982.
 33. Isogai, M, Shimada, N, Kamataki, T, Imaoka, S, and Funae, Y : Changes in the amounts of cytochrome P450 in rat hepatic microsomes produced by cyclosporin A. *Xenobiotica* **23** : 799-807, 1993.